

QUALITY ASSURANCE/QUALITY CONTROL
SM 9020, 9030, 9040, 9050 & 9060 (As published in SM 20th and 21st Editions)

Facility Name: _____ VELAP ID _____
 Assessor Name: _____ Analyst Name: _____ Inspection Date _____

Relevant Aspect of Standards	Method Reference	Y	N	N A	Comments
Thermometers					
1) Is the necessary calibration correction factor marked on each temperature measuring device so that only calibrated-corrected temperature values are recorded?	9020B.3.a.				
Balance					
2) Does the laboratory follow manufacturer's instructions in operation and routine maintenance of analytical and top-loading balances?	9020B.3.b				
3) Does the balance provide a sensitivity of at least 0.1 g at a load of 150 g, with weights traceable to appropriate national standards? Is an analytical balance used having a sensitivity of 1 mg under a load of 10 g for weighing small quantities (less than 2 g) of materials?	9020B.3.b. & 9030B.7				
4) Are working weights checked monthly against a set of reference weights of known tolerance (e.g., ANSI/ASTM Class 1 or NIST Class S accompanied by appropriate certificate) for accuracy, precision, and linearity?	9020B.3.b				
5) Is balance serviced annually or more often as conditions change or problems occur?	9020B.3.b				
pH Meter					
6) Is a meter used, graduated in ≤ 0.1 pH units, that includes temperature compensation?	9020B.3.c				
7) Is the pH meter calibrated before each use with at least two certified pH buffers that bracket the pH of sample being measured?	9020B.3.c				
8) Are buffer solutions discarded immediately after being used to calibrate meter?	9020B.3.c				
Mechanical media dispensing apparatus					
9) Is volume checked by dispensing into a graduated cylinder at the start of each volume change?	9020B.3 f				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
Autoclave					
10) Is glassware autoclaved at 121°C for at least 15 min? Are all bottle caps loosened before autoclaving?	9040				
Hot-air sterilizing oven (If needed, see referenced sections)	9020B.3.g & 9040				
Refrigerator					
11) Is temperature maintained at 1 to 4.4°C with thermometer bulb in distilled water or glycerol solution?	9020B.3.i & 9030B.11.				
Freezer (If needed, see referenced sections)	9020B.4.j)				
Membrane Filtration Equipment					
12) Are filter funnel and membrane holders made of seamless stainless steel, glass, or autoclavable plastic, and leak-proof and are not scratched or corroded?	9030B.15				
Incubator (air, water-jacketed, or aluminum block)					
13) Incubating Rooms: When culture incubation is conducted in an incubating room, is the room equipped with heating units, forced air circulation, and air exchange ports? Is temperature recorded in areas where plates or tubes are incubated? Are open metal wire or perforated shelves spaced to ensure temperature uniformity throughout the chamber? Is there a 2.5-cm space between walls and stacks of dishes or baskets of tubes?	9030B.1.				
14) Is temperature checked and recorded on the shelves in use?	9020B.3.o				
15) If a glass thermometer is used, is the bulb and stem submerged in water or glycerin to the immersion mark?	9020B.3.o				
Inoculating Equipment					
16) Are ≥3 mm diameter wire loops made of 22 or 24 gauge nickel alloy or platinum-iridium for flame sterilization used?	9030B.17				
17) Are prepackaged sterile disposable plastic loops or single-service hardwood or plastic applicators, 0.2 to 0.3 cm in diameter and at least 2.5 cm longer than the fermentation tube used?	9030B.17				
18) Are wooden applicators sterilized by dry heat and plastic applicators by autoclave, while stored in glass or other nontoxic containers?	9030B.17				

Notes/Comments:

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Dilution Water Bottles					
19) Are bottles or tubes closed with glass stoppers or screw caps equipped with non toxic liners?	9030B.13				
20) Are graduation levels marked indelibly on side of dilution bottles or tubes?	9030B.13				
21) Are plastic bottles of acceptable size substituted for glass provided that they can be sterilized properly?	9030B.13				
Dilution Water Blanks					
22) Is each batch or lot, checked at least one per batch or lot, or a set percentage. [e.g., 1 to 4%, for pH (7.2 ± 0.1) and volume (99±2 mL)]?	9020B.4.c				
23) Are dilution water bottles examined for a precipitate and discarded if present?	9020B.4.c				
Petri Dishes					
24) For HPC are 100 X 15 mm or 150 X 20 mm glass or plastic petri dishes used?	9030B.14				
25) Do dishes have bottoms that are free from bubbles and scratches and are flat so the medium will be of uniform thickness throughout the plate?	9030B.14				
26) For the membrane filter technique, are 60 X 15 mm loose-lid glass or plastic dishes, or 50 X 12 mm tight-lid dishes used?	9030B.14				
27) Are glass petri dishes sterilized and stored in metal cans (aluminum or stainless steel, but not copper), or wrapped in sulfate pulp (kraft) paper before sterilizing?	9030B.14				
Sample Bottles					
28) If preparing sample bottles in-house, use reusable bottles of glass or plastic made of nontoxic materials, such as polypropylene.	9030B.18				
Pipets, Micropipets, and Graduated Cylinders					
29) Is the error of calibration for a given manufacturer's lot no more than 2.5%?	9030B.9				
30) Is a pipet aid used and there is no pipetting by mouth?	9030B.9				

Notes/Comments:

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Washing And Sterilization					
31) Is all contaminated laboratory ware sterilized before cleaning?	9040				
32) Is stainless steel or other nontoxic material used for the rinse-water system? Is copper plumbing not used to distribute water?	9040				
33) Reagent-grade water Does reagent water quality for the microbiology laboratory meet the limits in Table 9020:II? <u>With Each Use</u> Conductivity <2 µmhos/cm or >0.5 megohms resistance at 25°C pH 5.5 – 7.5 <u>Monthly Tests:</u> Total organic carbon <1.0 mg/L Total chlorine residual <0.1 mg/L Heterotrophic plate count (SM 9215B) <500 CFU/mL Ammonia/organic nitrogen <0.10 mg/L <u>Quarterly tests</u> Use Test Student's $t \leq 2.78$ <u>Annual Tests</u> Single metals (Cd, Cr, Cu, Ni, Pb, and Zn) <0.05 mg/L Total metals <0.10 mg/L	9020B.4.d.				
Membrane filters and pads					
34) After sample incubation, are colonies well-developed with appropriate color and shape as defined by the test procedure? Does the gridline ink not channel growth along the ink line nor restrict colony development? Are colonies distributed evenly across the membrane surface?	9020B.4.h				
Culture media					
35) Are media ordered in quantities to last no longer than 1 yr?	9020B.4.i				
36) Are media used on a first-in, first-out basis?	9020B.4.i				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments														
37) Are dehydrated media stored in a tightly closed container in a cool, dry place away from direct sunlight?	9020B.4.i																		
38) Are opened bottles of media used within 6 months?	9020B.4.i																		
39) Are media prepared in clean containers that are at least twice the volume of the medium being prepared?	9020B.4.i.1)																		
40) After rehydrating a medium, are media dispensed promptly to culture vessels and sterilized within 2 h?	9050A.3.																		
41) Are media sterilized at 121°-124°C maximum for minimum time specified? <table><tr><td><u>Material</u></td><td><u>Time in min at 121°C</u></td></tr><tr><td>Membrane filters and pads</td><td>10</td></tr><tr><td>Carbohydrate-containing media (e.g. lauryl tryptose, BGB broth)</td><td>12–15</td></tr><tr><td>Contaminated materials and discarded cultures</td><td>30</td></tr><tr><td>Wrapped membrane filter assemblies and empty sample collection bottles</td><td>15</td></tr><tr><td>Buffered dilution water, 99 mL in screw-cap bottle</td><td>15</td></tr><tr><td>Rinse water, volume >100 mL</td><td>Adjust time for volume</td></tr></table>	<u>Material</u>	<u>Time in min at 121°C</u>	Membrane filters and pads	10	Carbohydrate-containing media (e.g. lauryl tryptose, BGB broth)	12–15	Contaminated materials and discarded cultures	30	Wrapped membrane filter assemblies and empty sample collection bottles	15	Buffered dilution water, 99 mL in screw-cap bottle	15	Rinse water, volume >100 mL	Adjust time for volume	9020B.4.i.2) & Table 9020:III				
<u>Material</u>	<u>Time in min at 121°C</u>																		
Membrane filters and pads	10																		
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Wrapped membrane filter assemblies and empty sample collection bottles	15																		
Buffered dilution water, 99 mL in screw-cap bottle	15																		
Rinse water, volume >100 mL	Adjust time for volume																		
42) Are media containing carbohydrates not exposed to the elevated temperatures for more than 45 min? Exposure time is defined as the period from initial exposure to heat to removal from the autoclave. Are autoclave printout records maintained?	9020B.4.i.2)																		
43) Are media not reautoclaved?	9020B.4.i.2)																		
44) Are melted agars tempered before use in a water bath 44 to 46°C, for ≤3 h? Is agar temperature monitored by exposing a bottle of water or medium containing a thermometer to the same heating and cooling conditions as the agar?	9020B.4.i.3)																		
45) Is unused liquid agar discarded and not allowed to harden or be remelted for later use?	9020B.4.i.3)																		
46) Are agar plates stored in sealed plastic bags or other sealed container if they will be held more than 2 d?	9020B.4.i.4)																		

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments														
47) Are media used within holding times below? <table><tr><td><u>Medium</u></td><td><u>Holding Time</u></td></tr><tr><td>Broth in tightly closed screw-cap flasks at 4°C</td><td>96 h</td></tr><tr><td>Poured agar in plates with tight-fitting covers at 4°C</td><td>2 weeks</td></tr><tr><td>Agar or broth in loose-cap tubes at 4°C</td><td>2 weeks</td></tr><tr><td>Poured agar plates with loose-fitting covers inverted in sealed plastic bags at 4°C</td><td>2 weeks</td></tr><tr><td>Agar or broth in tightly closed screw-cap tubes or other sealed containers</td><td>3 months</td></tr><tr><td>Large volume of agar in tightly closed screw-cap flask or bottle at 4°C</td><td>3 months</td></tr></table>	<u>Medium</u>	<u>Holding Time</u>	Broth in tightly closed screw-cap flasks at 4°C	96 h	Poured agar in plates with tight-fitting covers at 4°C	2 weeks	Agar or broth in loose-cap tubes at 4°C	2 weeks	Poured agar plates with loose-fitting covers inverted in sealed plastic bags at 4°C	2 weeks	Agar or broth in tightly closed screw-cap tubes or other sealed containers	3 months	Large volume of agar in tightly closed screw-cap flask or bottle at 4°C	3 months	9020B.4.i.4)& Table 9020:IV				
<u>Medium</u>	<u>Holding Time</u>																		
Broth in tightly closed screw-cap flasks at 4°C	96 h																		
Poured agar in plates with tight-fitting covers at 4°C	2 weeks																		
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Poured agar plates with loose-fitting covers inverted in sealed plastic bags at 4°C	2 weeks																		
Agar or broth in tightly closed screw-cap tubes or other sealed containers	3 months																		
Large volume of agar in tightly closed screw-cap flask or bottle at 4°C	3 months																		
48) Are prepared media containing dyes protected from light and excessive evaporation? If color changes occur, are media discarded?	9020B.4.i.4) & 9050A.1																		
49) Is the liquid level marked in several tubes after sterilization and monitored for lost weight or volume if stored for more than 2 weeks? If loss is 10% or more, is batch discarded? <i>NOTE: Requirement applies to sample test media, not QC media.</i>	9020B.4.i.4)																		
50) If media are refrigerated, are they incubated overnight at test temperature before use and the batch rejected if growth or false positive responses are present? <i>NOTE: Requirement may be fulfilled by incubating a media sterility blank with each batch of samples. Requirement applies to sample test media, not QC media.</i>	9020B.4.i.4)																		
51) Is a complete record of each batch of laboratory prepared medium maintained with date and name of preparer, name and lot number of medium, amount of medium weighed, volume of medium prepared , sterilization time and temperature, pH adjustments needed, final pH, and preparations of labile components?	9020B.4.i.5)																		
Dilution Water																			
52) Is stock phosphate buffer solution prepared as follows? Dissolve 34.0 g KH ₂ PO ₄ in 500 mL reagent-grade water, adjust to pH 7.2 ± 0.5 with 1N NaOH, and dilute to 1 L with reagent-grade water. Is stock solution discarded if turbidity develops?	9050C.1.a.																		

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
53) Is MgCl_2 stock solution prepared as follows? Add 81.1 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ to 1 L reagent grade water. Is stock solution discarded if solution becomes turbid?	9050C.1.a.				
54) Is dilution water working solution prepared as follows? Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride stock solution to 1 L reagent-grade water. Dispense in amounts that will provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min.	9050C.1.a.				
55) Is peptone water, 0.1%, prepared as follows? Prepare a 10% solution of peptone in distilled water. Dilute a measured volume to provide a final 0.1% solution. Final pH should be 6.8 after sterilization. Dispense in amounts to provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min.	9050C.1.b.				
56) Is sample suspended in dilution water for ≤ 30 min at room temperature?	9050C.1.b.				
Analytical Quality Control Procedures for Established Methods					
57) Are certified reference cultures in Table 9020:V used for testing media?	9020B.8.a.3) & Table 9020:V				
58) Duplicate analyses—Are duplicate analyses performed on 10% of samples and on at least one sample per test run? A test run is defined as an uninterrupted series of analyses. If the laboratory conducts less than 10 tests/week, are duplicate analyses performed on at least one sample each week?	9020B.8.a.4)				
59) Is precision of replicate analyses calculated for each different type of sample examined (drinking water, ambient water, or wastewater) according to the procedure outlined at SM 9020B.8.b?	9020B.8.b .				
Verification 9020B.9					
SM 9221B Total Coliform Multiple-tube fermentation (MTF) confirmation method 9020B.9.a.1)					
60) <u>Drinking Water</u> —Are tests carried through the confirmed phase?	9020B.9.a.1)a)				
61) <u>Drinking Water</u> - For QC purposes, if normally there are no positive results within a quarter, is at least one positive source water sample analyzed to confirm that the media and laboratory procedures and equipment produce appropriate responses?	9020B.9.a.1)a)				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
62) <u>Drinking Water</u> - For samples with a history of heavy growth without gas in presumptive-phase tubes, are the tubes carried through the confirmed phase to check for false negative responses for coliform bacteria? Are any positives verified for thermotolerant (fecal) coliforms or <i>E. coli</i> ?	9020B.9.a.1)a)				
63) <u>Other water types</u> —Is verification achieved by performing the completed phase on 10% of samples positive through the confirmed phase?	9020B.9.a.1)b)				
SM 9223B Total Coliform/<i>E. coli</i> confirmation method 9020B.a.9.2)					
64) <u>Drinking water</u> —Are at least 5% of total coliform positive results from enzyme substrate coliform tests verified by inoculating growth from a known positive sample and testing for lactose fermentation or for β -D-galactopyranosidase by the o-nitrophenyl- β -D-galactopyranoside (ONPG) test and indophenol by the cytochrome oxidase (CO) test? See 9225D for these tests. Coliforms are ONPG-positive and cytochrome-oxidase-negative.	9020B.9.a.2)a.				
65) Are <i>E. coli</i> positive DW tests verified using the EC MUG test (see 9221F)?	9020B.9.a.2)a				
66) <u>Other water types</u> —Are at least 10% of total coliform positive samples verified as in ¶ 2a above?	9020B.9.a.2)b				
SM 9230C Fecal Streptococci Multiple-tube fermentation (MTF) confirmation method 9020B.9.a.3)					
67) <u>Fecal streptococci procedure</u> - Is verification performed as outlined in Section 9230C.5?	9020B.9.a.3)				
Membrane Filter Verification Methods 9020B.9.b					
68) <u>Total coliform procedures in drinking water, SM9222B</u> : Are five typical and five atypical (nonsheen) colonies picked from positive samples on M-Endo medium and verified as in Section 9222B.5f.?	9020B.9.b.1)a)				
69) <u>Drinking Water</u> : Are any positive total coliform colonies verified for fecal coliforms OR <i>E. coli</i> as specified in SM9222B.4.f.	9020B.9.b.1)a)				
70) If there are no positive samples, is at least one known positive source water sample tested quarterly?	9020B.9.b.1)a)				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
71) <u>Total coliform procedures in water other than drinking water</u> : Are positives verified monthly by picking at least 10 typical and atypical colonies from a positive water sample as in Section 9222B.5f? Are counts adjusted based on percent verification?	9020B.9.b.1)b)				
72) Are false negatives determined in any water type by picking representative atypical colonies of different morphological types and verifying as in Section 9222B.5f?	9020B.9.b.1)c)				
73) <u>Fecal coliform procedure (SM 9222D)</u> – Are positives verified monthly by picking at least 10 blue colonies from one positive sample using lauryl tryptose broth and EC broth as in Sections 9221B.1 and 9221E? Are counts adjusted based on percent verification?	9020B.9.b.2)a)				
74) Are false negatives determined by picking representative atypical colonies of different morphological types and verifying as in 9221B.2 and 9221E?	9020B.9.b.2)b)				
75) <u>Escherichia coli procedure (9222G)</u> Drinking water—Are at least 5% of MUG-positive and MUG-negative results verified as follows?. Pick from well-isolated sheen colonies that fluoresce on nutrient agar with MUG (NA-MUG), taking care not to pick up medium, which can cause a false positive response. Also verify nonsheen colonies that fluoresce. Verify by performing the citrate test and the indole test as described in 9225D, but incubate indole test at 44.5°C. <i>E. coli</i> are indole positive and yield no growth on citrate.	9020B.9.b.3)a)				
76) <u><i>E. coli</i> procedure for water other than drinking water</u> - Is one positive sample verified monthly by the procedure described above in 9020B.9.b.3)a)? Are counts adjusted according to percentage of verification?	9020B.9.b.3)b)				
77) <u>Fecal streptococci procedure</u> : At least monthly, are at least 10 isolated esculin-positive red colonies from m-Enterococcus agar picked to brain heart infusion (BHI) media and tested as described in Section 9230C? Are counts adjusted according to percentage of verification?	9020B.9.b.4)				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
78) <i>Enterococci</i> procedures - Are at least 10 well-isolated pink to red colonies with black or reddish-brown precipitate from EIA agar picked to verify monthly and transfer to BHI media as described in 9230C? Are counts adjusted according to percentage of verification?	9020B.9.b.5)				
Samples 9060					
79) Are samples rejected if sample bottles do not have at least 2.5 cm of air space in the bottle and resampling requested? Alternatively, are overfilled samples added to a larger sterile sample bottle in the laboratory to assure adequate mixing?	9060A.3				
80) <i>Drinking water for compliance purposes</i> : For total coliform and <i>E. coli</i> analyses, is the holding time from collection to analysis no more than 30 h?	9060B.1.b				
81) Are samples for heterotrophic plate count analysis maintained at <8°C and do not exceed 8 h holding time?	9060B.1.b				

Notes/Comments: